Korean J. Food Sci. Ani. Resour.
Vol. 32, No. 3, pp. 316~322(2012)
DOI http://dx.do.org/10.5851/kosfa.2012.32.3.316

ARTICLE

Effects of Soaking pH and Extracting Temperature on the Physicochemical Properties of Chicken Skin Gelatin

Hyun-Wook Kim, Dong-Heon Song, Yun-Sang Choi[1], Hack-Youn Kim[2], Ko-Eun Hwang, Jae-Hyun Park, Yong-Jae Kim, Ji-Hun Choi[3], and Cheon-Jei Kim*

Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, Korea
[1]Food and Biological Resources Examination Division, Korean Intellectual Property Office, Daejeon 302-701, Korea
[2]Department of Animal Resources Science, Kongju National University, Yesan 340-702, Korea
[3]Food and Industrial Ingredients Center, CJ CheilJedang, Seoul 152-051, Korea

Abstract

This study was conducted to evaluate the effects of soaking pH and extraction temperature on the physicochemical properties of chicken skin gelatin. In order to extract gelatin from chicken skin, the chicken skin was soaked at various pH ranges (1-13) and was extracted at 75 and 100°C. For the rate of weight increase, the highest value was obtained from two pH ranges (1-2 and 12-13). In addition, the rate of weight increase was affected by soaking time. The alkali treatments had greater crude protein content as well as total extraction yield compared to the acid process ($p<0.05$), and the increased extraction temperature resulted in a significant ($p<0.05$) increase of crude protein content and total extraction yield. All treatments showed α 1 and α 2 chains derived from type I collagen on SDS-PAGE. The pH value and color of gelatin gel (6.67%) were affected by soaking pH and extraction temperature. Chicken skin gelatin gel extracted at 75°C after soaking at a pH of 2 had the highest melting point ($p<0.05$) and gel strength among all treatments. Although the chicken skin treated with the alkali process had a higher yield, a lower extraction temperature following the acid process would be better for obtaining superior gelatin from chicken skin.

Key words: collagen, gelatin, chicken skin, by-products of chicken

Introduction

Collagen, which is obtained from skin, bone, cartilage, ligament, blood vessel, and connective tissue of animal, is a fibrous structural protein. The standard unit of collagen includes a triple helix structure that is based on tropocollagen. The amino acid composition may differ slightly depending on the kinds of animals and its parts, but glycine, proline, and hydroxyproline are the most common components (Kim *et al*., 2010). Recently, collagen has been used in a wide range of industrial fields, including medicine, medical supplies, and cosmetic products, due to its anti-aging, antihypertensive, and carcinostatic effects. Moreover, collagen has been used as functional materials in the food industry due to the ability to formation of a gel and emulsion and its water binding capacity (Gómez-Guillén *et al*., 2011). Collagen used for commercial applications is generally obtained from pork skin, cowhide, and fish scales (Kim *et al*., 2010; Shon and Eun, 2010). Recently, Kim *et al*. (2010) reported that collagen extracted from cow and pork can transfer bovine spongiform encephalopathy (BSE) and foot-and-mouth disease. Thus, advanced research is needed to develop new extraction materials and extraction methods to improve food safety and effective use of animal resources.

By-products of chicken are a potentially excellent source of collagen, studies about the properties and extraction conditions of collagen extracted from chicken feet have been conducted (Jang *et al*., 2002; Lim *et al*., 2002; Shin, 2002). Chicken skin which contains approximate 3% collagen has also been identified as a potential novel collagen source (Bonifer and Froning, 1996). Cliche *et al*. (2003) reported that the collagen extracted from chicken skin is comprised of about 75% type I collagen and 15% type III collagen. Extraction methods of collagen from chicken skin involve acetic acid and pepsin treatment (Bannister and Burns, 1972) and pepsin or ethylene diamine extraction after mechanical separation (Cliche *et al*., 2003). Gelatin derived from the collagen through

*Corresponding author: Cheon Jei Kim, Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, Korea. Tel: 82-2-450-3684, Fax: 82-2-444-6695, E-mail address: kimcj@konkuk.ac.kr

hydrolysis or the thermal denaturation is widely used as an emulsifying agent, a viscosity agent, a stabilizer, and a gelling agent in various foods (Bailey and Paul, 1998; Yeom *et al*., 2004). Differences in the extraction conditions have been shown to affect the characteristics and shape of gelatin gel. Thus, extraction conditions are the most important factors to determine gelatin gel properties (Kim *et al*., 1988). However, the effects of different extraction conditions on the properties of chicken gelatin gel have not yet been evaluated.

Therefore, the objective of this study was to evaluate the effects of soaking pH (1-13) and extraction temperatures (75 and 100°C) on the yields and physicochemical properties of chicken skin gelatin gel, and to produce valuable gelatin using by-products of chicken.

Materials and Methods

Extraction of chicken skin collagen

A total of 52 fresh broiler carcasses (Arbor acre strain), ranging in weight from 800 to 900 g (average live weights: 1.6±0.2 kg), were obtained from the local municipal slaughterhouse. The carcasses were washed and the skin was immediately removed. Visible subcutaneous fat was then removed. Approximately 1.4 kg of skin was obtained and washed in distilled water. The skins were cut into 3×3 cm pieces and collagen was extracted. The extraction procedures were shown in Fig. 1. The manicured chicken skins after weighing were soaked in different pH solutions (1-13), which were adjusted using 0.1 N HCl and 0.1 N NaOH solutions, with 10 volumes (v/w) at 15°C for 24 h. After the soaking, the chicken skin was washed in running water to adjust the pH within between pH 5 to 7 (neutralizing processing) to minimize decline of gel strength. After the neutralizing processing, skins were placed in polyethylene bags (FoodSaver®, Korea) and vacuum packaged using a vacuum packaging system (FJ-500XL, Fujee Tech, Korea). The packaged skin samples were heated at 75 and 100°C for 1 h in a boiling water bath (Model 10-101, Dae Han Co., Korea). The extract in polyethylene bags were then filtered through nylon filter and stored in a 4°C refrigerator for 6 h. The top layer of fat was removed, and the gelatin gel was frozen in a -70°C deep freezer (DF8715, Ilshin Lab Co., Korea). The frozen gelatin was freeze-dried at -40°C under a pressure of 80×10^{-3} torr using a freeze-dryer (PVTFD20R, Ilshin lab., Korea). The gelatin powder was vacuum-packaged in polyethylene bag and stored in a -20°C refrigerator until analysis. The gelatin powder was dissolved with 40°C distilled water (6.67%, w/v) for 1 h until completely dispersed and then was gelated at 4°C for 6 h prior to analysis.

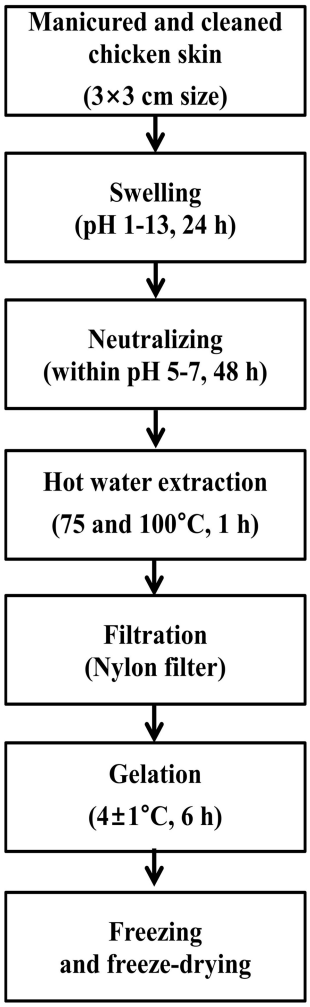

Fig. 1. Procedures for preparation of the gelatin powder from the chicken skins.

Rate of weight increase (swelling)

The rate of weight increase was determined by calculating the weight differences of sample before and after swelling at 6, 12, and 24 h and after neutralizing processing (24+48 h). The expression is as follows;

$$\text{Rate of weight increase (\%)} = \frac{\text{Weight of sample after soaking (g)}}{\text{Wet weight of raw material (g)}} \times 100$$

Gelatin gel and total extraction yields

Gelatin gel and gelatin powder yields (total extraction yields) were determined by calculating the weight differences of sample before and after processing as follows:

Gelatin gel yield (%)

$$= \frac{\text{Weight of gelatin gel (g)}}{\text{Wet weight of raw material (g)}} \times 100$$

Total extraction yield (%)

$$= \frac{\text{Dry weight of gelatin powder (g)}}{\text{Wet weight of raw material (g)}} \times 100$$

Proximate composition

The proximate composition of the gelatin gel was determined using AOAC (2000) procedures. Moisture content (950.46B, oven air-drying method) was determined by weight loss after 12 h of drying at 105°C in a drying oven (SW-90D, Sang Woo Scienctific Co., Korea). Fat content (960.69, ether extractable component) was determined by the Soxhlet method with a solvent extraction system (Soxtec® Avanti 2050 Auto System, Foss Tecator AB, Sweden), and protein content (981.10) was determined by the Kjeldahl method with an automatic Kjeldahl nitrogen analyzer (Kjeltec® 2300Analyzer Unit, Foss Tecator AB, Sweden).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE of the collagen form chicken skin was performed by the method of Laemmli (1970), using 8% running gels and 5% stacking gels. The loaded gel was stained with Coomassie Brilliant Blue R250 (B7920, Sigma, USA), and was destained in methanol : distilled water : acetic acid (50:40:10). The separated protein bands were identified by comparison with those of standard protein marker (Precision Plus Protein Standards, Bio-Rad Lab., USA), which included 250, 150, 100, 75, 50, 37, 25, 20, 15, and 10 kDa bands.

pH measurements

The pH values of gelatin gel (6.67% concentration) were determined with a pH meter (Model 340, Mettler-Toledo GmbH, Switzerland). The pH values of samples were measured by blending a 5 g sample with 20 mL distilled water for 60 s in a homogenizer at 8,000 rpm (Ultra-Turrax SK15, Janke & Kunkel, Germany) (Choi *et al*., 2011).

Instrumental color evaluation

Instrumental color of gelatin gel (6.67% concentration) was determined using a colorimeter (Minolta Chroma meter CR-210, Japan; illuminate C, calibrated with a white plate, CIE L^*=+97.83, CIE a^*=-0.43, CIE b^*=+1.98). Five measurements for each of five locations on surface of gelatin gel were taken. CIE L^* (lightness), CIE a^* (redness), and CIE b^* (yellowness) values were recorded.

Melting point

The melting point of gelatin gel (6.67% gelatin concentration) was determined by average temperature between starting melting and ending melting temperatures using melting point apparatus analyzer (ATM-01, AS ONE, Japan) (Kim *et al*., 1988).

Gel strength

The gel strength was determined according to the AOAC (2000) procedures. The gel strength of 6.67% gelatin gel was measured under following conditions; plunger, 12.7 mm diameter penetration depth, 4 mm; and penetration speed, 2 cm/min.

Statistical analysis

An analysis of variance was performed on all the variables measured using the General Linear Model (GLM) procedure of the SAS statistical package (SAS Institute, Inc., 2008). Duncan's multiple range test (p<0.05) was used to determine differences between treatment means.

Results and Discussion

Swelling and neutralizing processing

The changes in the rate of weight increase of the chicken skin soaked in different pH solutions (pH 1-13) were shown in Fig. 2. Generally, the acid process is used to extract pork and fish collagens, and the alkali process is mainly used with bovine hide (Jang *et al*., 2002). The acid or alkali processes are typically used for collagen extraction, because these processes undermine the cross-linked collagen molecules (Kim *et al*., 1988). The rate of weight increase is a good indicator of the degree of swelling. The rate of weight increase in the chicken skin was significantly different at the three different pH ranges (pH 1-2, 3-11, and 12-13), and the chicken skin soaked at pH 2 had the highest rate of weight increase. Asghar and Henrickson (1982) reported that an increase in the hydrogen ion concentration with the addition of acid (acid process) restricts the effects of the negative ions on the collagen molecules, whereas the hydroxide ion (alkali process) inhibited the positive ion. Moreover, this elimination of electrical force with acid or alkali treatments facilitates the extraction of collagen due to structural changes in the collagen protein. Similarly, Kim *et al*.

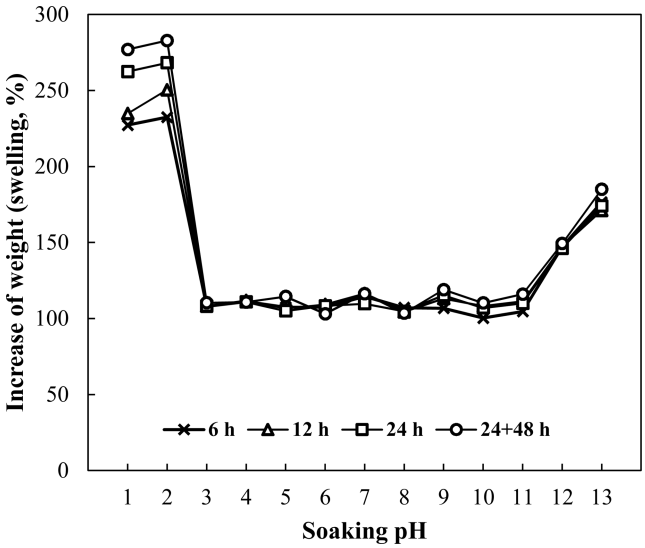

Fig. 2. Changes in the increase of weight of chicken skin at various soaking pH and soaking time. 6, 12, and 24 h, soaking time; 24+48 h, soaking and neutralizing time.

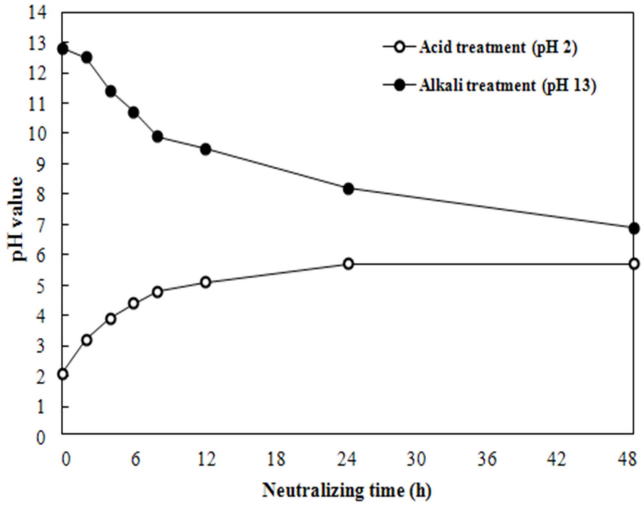

Fig. 3. Changes in the pH value of soaking solution during acid or alkali processing.

(1988) reported the lowest increase of weight in pork skin soaked at pH 4-9 and, suggested that the results was associated with the isoelectric point of raw collagen which is located between pH 4 and 7.5.

The weight of the soaked chicken skins increased with passing soaking time. The neutralizing process increased the rate of weight increase of the chicken skin. Neutralizing times of 24 h (acid treatment) and 48 h (alkali treatment), were needed when the pH of the soaking solution was 5.7 and 6.9, respectively (Fig. 3). According to Gómez-Guillén *et al*. (2011), under alkali process and additional acid process during neutralization, excessive salt is formed. In this study, running water was used during neutralizing process without an additional acid process; however, complete neutralization was difficult after alkali treatment. Also, the alkali treatments required a longer neutralization time than the acid treatment. Yeom *et al*. (2004) indicated that the acid process is generally used in gelatin extraction due to the breakdown of peptide bond and the complex neutral reaction.

The yields and proximate composition of gelatin gel

The effects of soaking pH and extraction temperatures on the yields and proximate composition of chicken skin gelatin gel were shown in Table 1. The highest gelatin gel yield and protein content were obtained at 100°C extraction after soaking in pH 13 (13/100) ($p<0.05$); however, there was no significant difference in protein content between alkali treatments ($p>0.05$). The gelatin gel extracted at 75°C after soaking in pH 2 (2/75) showed the highest moisture content among all treatments ($p<0.05$). The fat content for all treatments was below 0.08% (data not shown) and there were no significant ($p>0.05$) differences among the all treatments. The total extraction yields were measured to evaluate total solids content. As was observed for the protein content, alkali treatments resulted in significantly ($p<0.05$) higher total solid content than acid treatments. Also, an increased extraction temperature resulted in an increase in total extraction yield for both

Table 1. The effects of extraction conditions on the yields and proximate composition of chicken skin gelatin gel

Traits	Extraction conditions (soaking pH/extraction temperature, °C)			
	2/75	2/100	13/75	13/100
Gelatin gel yield[2)] (%)	42.80±1.96[1)C]	52.20±1.74[B]	50.90±2.20[B]	55.25±1.58[A]
Protein content (%)	2.57±0.05[C]	3.00±0.04[B]	4.96±0.19[A]	5.08±0.48[A]
Moisture content (%)	97.07±0.14[A]	96.22±0.11[B]	94.74±0.30[C]	94.52±0.14[C]
Total extraction yield[3)] (%)	1.74±0.22[D]	2.03±0.10[C]	3.88±0.18[B]	4.43±0.24[A]

[1)]All values are mean±SD of three replicates.
[2)]Gelatin gel yield, the difference in weight between wet raw chicken skin and gelatin gel.
[3)]Total extraction yield, the difference in weight between wet raw chicken skin and freeze-dried gelatin powder.
[A-D]Means within a row with different letters are significantly different ($p<0.05$).

acid and alkali treatments. Lim *et al*. (2002) reported similar results, and found that the yields for gelatin extracted from chicken feet increased with increasing extraction temperature. Divakaran (1984) reported that type B was obtained from the alkali process when cowhides were used. In this study, the method used to destroy the cross-linked collagen was shown to affect the gelatin gel and total extraction yields.

SDS-PAGE analysis

The SDS-PAGE electrophoresis is wildly used to identify the collagen chain (Cao and Xu, 2008); thus, this method was used to identify protein patterns of collagen extracted from chicken skin under various extraction conditions (Fig. 4). All treatments produced similar migration bands and contained two different main bands that corresponded to α 1 and α 2 chains derived from type I collagen, which have approximate molecular weights ranging from 120 to 130 kDa (Shin, 2002). In addition, the putative higher molecular weight band of the β chain (200 kDa) was observed in all treatments (Nalinanon *et al*., 2008). Thus, these results indicated that type I collagen was the major collagen type in chicken skin. Abedin and Riemschneider (1984) reported that chicken skin contains 75% type I collagen and 15% type III collagen. Similarly, Cliche *et al*. (2003) reported that chicken skin collagen treated with pepsin or ethylene diamine contains two α chains and the migration patterns of two treatments were similar. In addition, Shin (2002) found that the collagen in chicken feet was comprised of α 1 and α 2 chains. The alkali treatments showed significant low molecular weight fragments when compared to acid treatments. This result further demonstrates that the alkali process had a greater effect on weakening the cross-linked collagen than the acid process. However, Jang *et al*. (2002) reported that acid treatment produced higher amounts of low molecular weight fragments than alkali treatment for chicken feet. No differences in protein patterns were observed between extraction temperatures in the acid treatment; however, different proteins patterns were observed for the alkali treatments (13/75 and 13/100) in the region of 75 kDa. This was most likely due to the formation of low molecular weight fragments at the higher extraction temperature. Lin and Liu (2006) observed higher amounts of lower molecular weight fragments when the digestion temperature increased due to a loss of integrity.

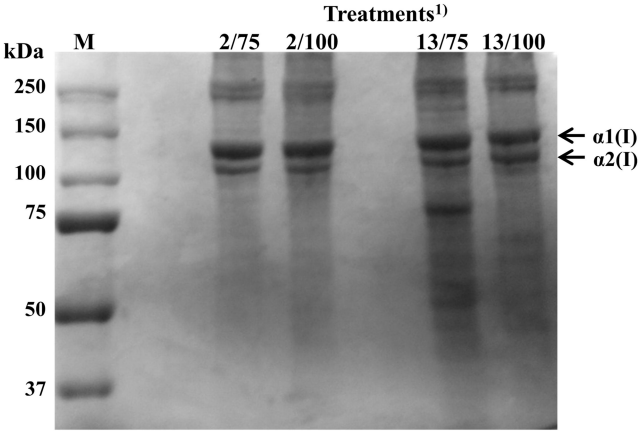

Fig. 4. Electrophoretogram (SDS-PAGE analysis) of the chicken skin collagen. 1)Treatment: 2/75, soaking pH 2/extraction temperature 75°C; 2/100, soaking pH 2/extraction temperature 100°C; 13/75, soaking pH 13/extraction temperature 75°C; 13/100, soaking pH 13/extraction temperature 100°C; line M, Standard protein marker.

pH and instrumental color of chicken skin gelatin gel

The freeze-dried gelatin powder was produced to evaluate the physicochemical properties of gelatin gel extracted from chicken skin under various conditions at an identical gelatin concentration of 6.67%. The pH value and instrumental color of the gelatin gel were shown in Table 2. The acid treatments had a significant (p<0.05) lower pH value than alkali treatments, however, there were no differences in the pH values when different extraction temperatures were used in the same soaking pH (p>0.05). The higher pH value of alkali treatments resulted in a higher ultimate pH at the end of the neutralizing process.

Table 2. The effects of extraction conditions on pH value and instrumental color of gelatin gel[1)] prepared with chicken skin

Traits	Extracting conditions (soaking pH/extraction temperature, °C)			
	2/75	2/100	13/75	13/100
pH	6.32±0.03[2)B]	6.33±0.04[B]	6.75±0.06[A]	6.78±0.04[A]
CIE L*	40.70±0.95[C]	43.83±1.40[B]	40.67±1.84[C]	46.75±0.99[A]
CIE a*	-0.34±0.05[B]	-0.67±0.06[C]	-0.20±0.06[A]	-0.35±0.02[B]
CIE b*	2.40±0.21[A]	0.52±0.40[B]	-2.83±0.51[C]	-5.32±0.70[D]

[1)]gelatin gel: 6.67% gelatin concentration (w/v).
[2)]All values are mean ± standard deviation of three replicates.
[A-D]Means within a row with different letters are significantly different (p<0.05).

The pH values of gels extracted from bovine and porcine treated with alkali solution (1-3% NaOH) and neutralized with 6 N HCl were 5.9 and 4.7, respectively (Cho *et al*., 2005).

The 13/100 treatment showed the highest lightness (CIE L^*) ($p<0.05$), and an increased extraction temperature resulted in an increase in the lightness for both the acid and alkali process. For redness (CIE a^*), alkali treatments had a higher value than acid treatments at the same extraction temperature ($p<0.05$) and an increased extraction temperature decreased the redness. The 2/75 treatment had the highest yellowness value (CIE b*) among all treatments ($p<0.05$), and the yellowness value decreased with increasing the soaking pH and extraction temperature. Similarly, Lim *et al*. (2002) reported that an increase in extraction temperature and time resulted in an increase in lightness and decrease in yellowness of chicken feet gelatin gel. However, Jang *et al*. (2002) reported that chicken feet gelatin gel treated with acid had a higher lightness and lower redness and yellowness compared to samples treated with alkali.

Melting point and gel strength of chicken skin gelatin gel

Melting point and gel strength of gelatin are major factors dictating the quality of gelatin (Cho *et al*., 2005). The effects of extraction conditions on melting point and gel strength of chicken skin gelatin gel were shown in Table 3. Generally, the gelatin had thermal reversible properties, and the stability of the gelatin gel was evaluated by measuring the melting point. Samples treated with acid treatments had higher melting points ($p<0.05$) than samples subjected to alkali treatments regardless of extraction temperature. The highest melting point value (39.83°C) was observed for the 2/75 treatment. The melting points of bovine and porcine were previoulsy reported by Cho *et al*. (2005) and Gudmundsson (2002) to be 33.8 and 36.5°C, 29.7 and 32.3°C, respectively. Cho *et al*. (2005) indicated that the difference in melting point was associated with the heat rate. In our study, the melting points of chicken skin gelatin at a heat rate of 1°C/min ranged from 36.88 to 39.83°C regardless of extraction conditions and these values were relatively high, when compared to the melting point of mammalian collagen.

Gel strength, which significantly influences consumer preference (Yeom *et al*., 2004), is affected by the collagen source, concentration of soaking solution, and soaking time (Jang *et al*., 2002). The gel strength of tuna, bovine, and porcine gelatin were previously reported to be 426, 216, and 295 g, respectively (Cho *et al*., 2005). The gel strength of chicken skin gelatin gel ranged from 217 to 270 g and the 2/75 treatment had the highest gel strength ($p<0.05$) of all treatments. An increase in soaking pH and extraction temperature decreased the gel strength. These results were associated with the loss of cross-linking and the increase in low molecular weight fragment concentrations. Lim *et al*. (2002) reported that an increase in extraction time and temperature resulted in a decreased in the hardness of chicken feet gelatin gel, and suggested that the extraction temperature should be within 40-45°C to prevent damage to the protein. Johnston-Banks (1990) found that the gel strength increased with an increase in the concentration of the higher molecular components. Thus, in this study, the lower gel strength for alkali treatments was most likely due to the low molecular weight fragments (Fig. 4), while an increase in extraction temperature resulted in a decrease of gel strength regardless of soaking pH.

In conclusion, the alkali process resulted in a higher crude protein content and total extraction yield. At identical gelatin concentrations (6.67%), the chicken skin gelatin gel that was subjected to the acid process had a higher melting point and gel strength. When considering the time required for neutralization after the alkali process and the physicochemical properties of gelatin gel, the acid process and lower extraction temperature would be optimal for obtaining superior gelatin from chicken skin.

Table 3. The effects of extraction conditions on melting point and gel strength of gelatin gel[1)] prepared with chicken skin

Traits	Extracting conditions (soaking pH/extraction temperature, °C)			
	2/75	2/100	13/75	13/100
Melting point (°C)	39.83±0.25^{A}	38.88±0.48^{B}	37.70±0.27^{C}	36.88±0.25^{D}
Gel strength (g)	270.5±2.4^{A}	252.8±3.5^{B}	237.8±6.6^{C}	217.8±4.4^{D}

[1)]gelatin gel: 6.67% gelatin concentration (w/v).
[2)]All values are mean ± standard deviation of three replicates.
[A-D]Means within a row with different letters are significantly different ($p<0.05$).

Acknowledgements

This study was supported by Technology Development Program (608001-05-3-SB310) for Agriculture and Forestry, Ministry for Agriculture, Forestry and Fisheries, Republic of Korea. This article was partially supported by the Brain Korea (BK21) Project from Ministry of Education and Human Resources Development, Republic of Korea.

References

1. Abedin, M. Z. and Riemschneider, R. (1984) Chicken skin collagen-molecular diversity and susceptibility to neutral proteinases. *Pharm. Ind*. **46**, 532-535.
2. AOAC. (2000) Official methods of analysis of AOAC Int1. 17th ed. Associations of Official Analytical Chemists, USA.
3. Asghar, A. and Henrickson, R. L. (1982) Chemical, biochemical and nutritional characteristics of collagen in food systems. *Adv. Food Res.* **28**, 231-372.
4. Bailey, A. J. and Paul, R. G. (1998) Collagen-is not so simple protein. *J. Soc. Leather Technol. Chem*. **82**, 104-110.
5. Bannister, D. W. and Burns, A. B. (1972) Pepsin treatment of avian skin collagen. Effects on solubility, subunit composition and aggregation properties. *Biochem. J*. **129**, 677-681.
6. Bonifer, L. J. and Froning, G. W. (1996) Chicken skin composition as affected by aqueous washings. *J. Food Sci*. **61**, 895-898.
7. Cao, H. and Xu, S. Y. (2008) Purification and characterization of type II collagen from chick sterna cartilage. *Food Chem*. **108**, 439-445.
8. Cho, S. M., Gu, Y. S., and Kim, S. B. (2005) Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. *Food Hydrocol.* **19**, 221-229.
9. Choi, Y. S., Choi, J. H., Han, D. J., Kim, H. Y., Lee, M. A., Kim, H. W., Jeong, J. Y., and Kim, C. J. (2011) Effects of rice bran fiber on heat-induced gel prepared with pork salt-soluble meat proteins in model system. *Meat Sci*. **88**, 59-66.
10. Cliche, S., Amiot, J., Averzard, C., and Gariépy, C. (2003) Extraction and characterization of collagen with or without telopeptides from chicken skins. *Poultry Sci*. **82**, 503-509.
11. Divakaran, S. (1984) Handbook of mammalian collagen and gelatin. *Biochem.* **61**, 589-585.
12. Gómez-Guillén, M. C., Gilménez, B., López-Caballero, M. E., and Montero, M. P. (2011) Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocol.* **25**, 1813-1827.
13. Gudmundsson, M. (2002) Rheological properties of fish gelatins. *J. Food Sci*. **67**, 2172-2176.
14. Jang, E. G., Lim, J., and Kim, K. O. (2002) Effect of soaking condition on the physicochemical properties of chicken feet gelatin. *Korean J. Food Sci. Technol*. **34**, 425-430.
15. Johnston-Banks, F. A. (1990) Gelatin. In: Food Gels. Harris, P. (ed) Elsevier Applied Food Science Series, London, pp. 223-289.
16. Kim, C. J., Kim, K. H., and Choe, B. K. (1988) Effect of pH, swelling temperature, swelling time and various acids on the yields and physicochemical properties of pigskin gelatin gel. *Korean J. Anim. Sci*. **30**, 301-306.
17. Kim, J. W., Kim, D. K., Kim, M. J., and Kim, S. D. (2010) Extraction and bleaching of acid- and pepsin-soluble collagens from shark skin and muscle. *Korean J. Food Preserv.* **17**, 91-99.
18. Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.
19. Lim, J., O, S., and Kim, K. O. (2001) The effects of processing conditions on the properties of chicken feet gelatin. *Food Sci. Biotechnol*. **10**, 638-645.
20. Lim, J., Shin, W. S., Lee, H. G., and Kim, K. O. (2002) Optimizing extraction conditions for chicken feet gelatin. *Korean J. Food Sci. Technol*. **34**, 824-829.
21. Lin, Y. K. and Liu, D. C. (2006) Effects of pepsin digestion at different temperatures and times on properties of telopeptide-poor collagen from bird feet. *Food Chem*. **94**, 621-625.
22. Nalinanon, S., Benjakul, S., Visessanguan, W., and Kishimura, H. (2008) Tuna pepsin: characteristics and its use for collagen extraction from the skin of threadfin bream (*Nemipterus* spp.). *J. Food Sci*. **73**, C413-C419.
23. SAS. (2008) SAS/STAT Software for PC. Release 9.2, SAS Institute Inc., Cary, NC, USA.
24. Shin, M. H. (2002) Properties of collagen extracted from chicken foot skins. *Korean J. Culinary Res.* **8**, 95-105.
25. Shon, J. and Eun, J. B. (2010) Physicochemical and functional properties of collagen powder from skate (*Raja Kenojei*) skins. *Korean J. Food Preserv.* **17**, 435-443.
26. Yeom, G. W., Andrieu, J., and Min, S. G. (2004) Effect of acid treatment process on the physicochemical properties of gelatin extracted from pork skin. *Korean J. Food Sci. Ani. Resour*. **24**, 266-272.

(Received 2011.11.4/Revised 2012.4.19/Accepted 2012.6.12)